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## **Supplemental Information**

### **Regulation of Meristem Morphogenesis**

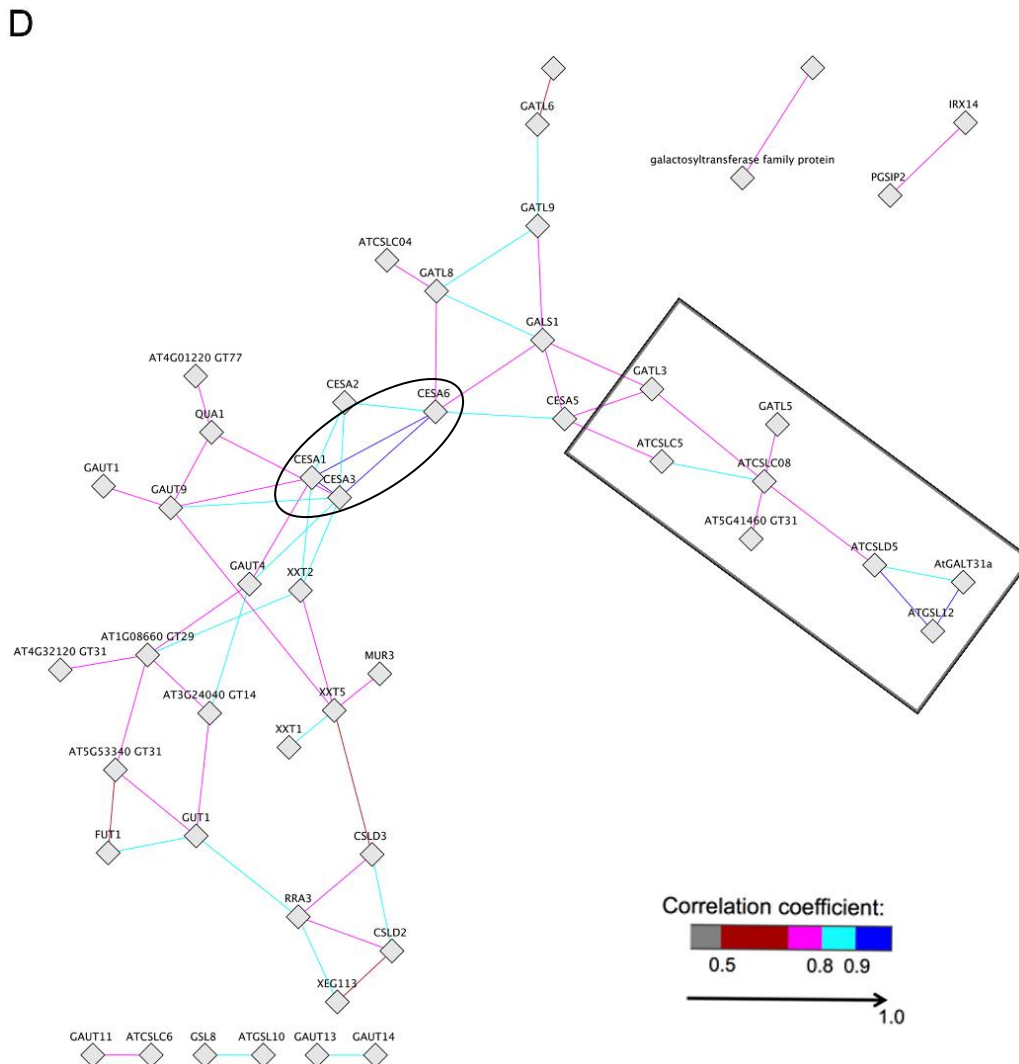
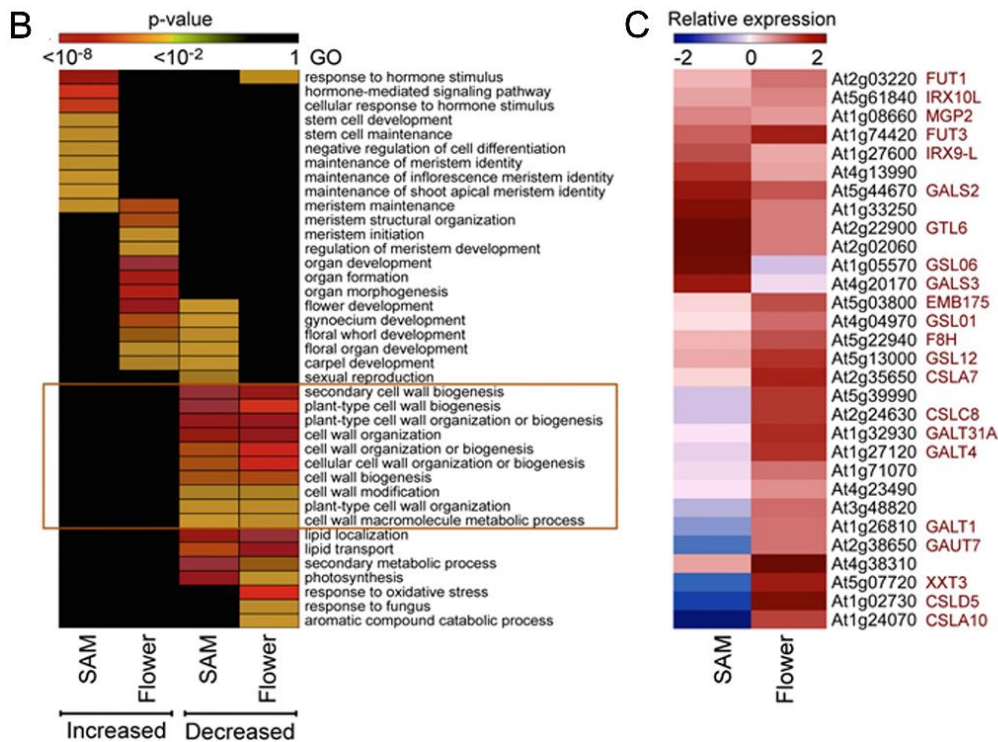
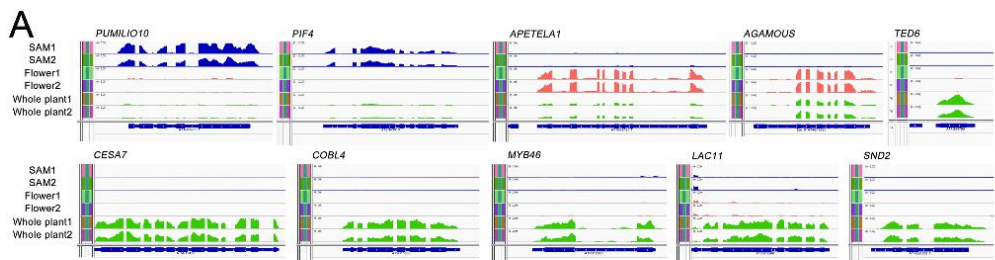
#### **by Cell Wall Synthases in *Arabidopsis***

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**Figure S1. The morphology of shoot apical meristems in wild-type and *clavata* mutants. Related to Figure 1.**

Top panels show an overview of the shoot apex in different genetic backgrounds and a close-up of the SAMs are shown in the bottom panels. Scale bar in top panel = 2 mm, in bottom panel = 0.2 mm.



**Figure S2. RNA-Seq reveals meristem-specific expression pattern of cell wall genes. Related to Figure 3.**

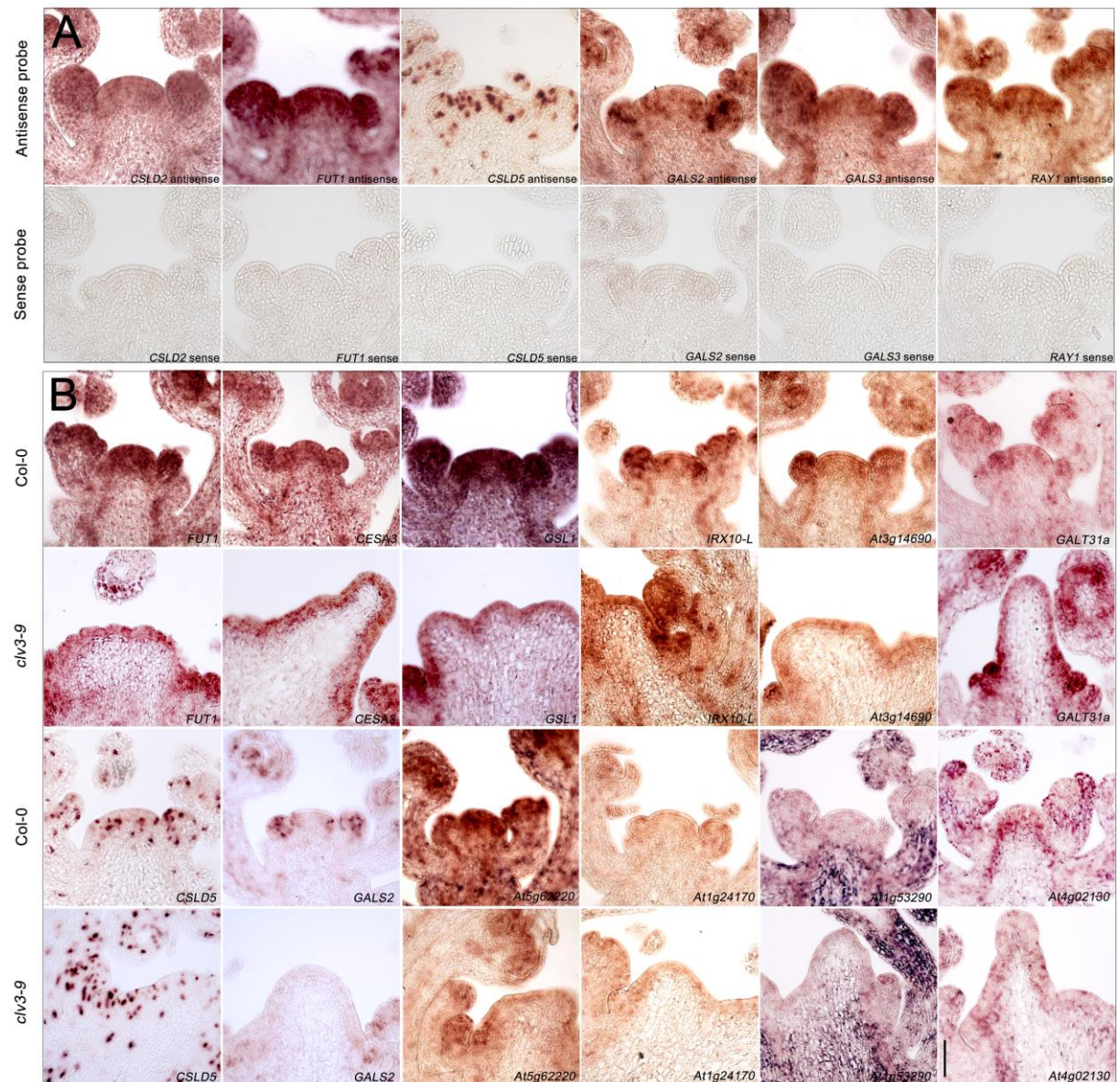
(A) Reads from RNA-seq mapped to individual genes with enriched expression in SAM, flower or differentiated tissues.

(B) Gene ontology (GO) term enrichment of genes with increased or decreased transcript abundance in SAM or flower compared to their average expression in the whole plant. Red indicates level of enrichment. Cell wall-related categories are boxed. A selection of GO terms is shown.

(C) Relative expression of differentially expressed GT family genes in SAM and flower ( $FC > 1.5$ ;  $P < 0.05$ ). Red indicates higher, and blue indicates lower expression than average expression in the whole plant. Names of characterized genes are given in red.

(D) Network of GT genes after pairwise correlation coefficient analysis using CORNET ([https://bioinformatics.psb.ugent.be/cornet/versions/cornet\\_maize1.0/main/precalt](https://bioinformatics.psb.ugent.be/cornet/versions/cornet_maize1.0/main/precalt)). The highly correlated core part of the network, encompassing members of the *CESA* family, is circled. A floral subnetwork is highlighted (boxed).

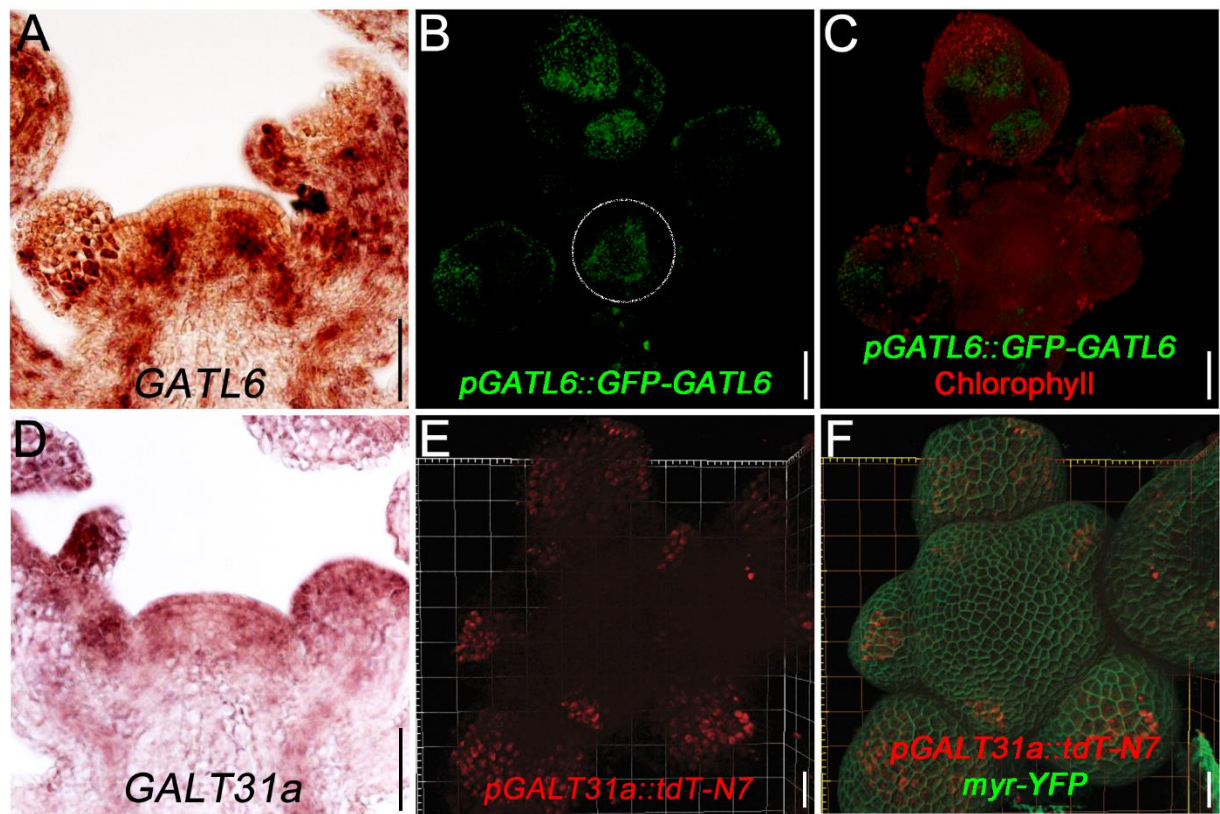




**Figure S3. *In situ* hybridization with RNA sense probes and comparison of GT gene expression in wild-type and *clv3* mutant. Related to Figure 3.**

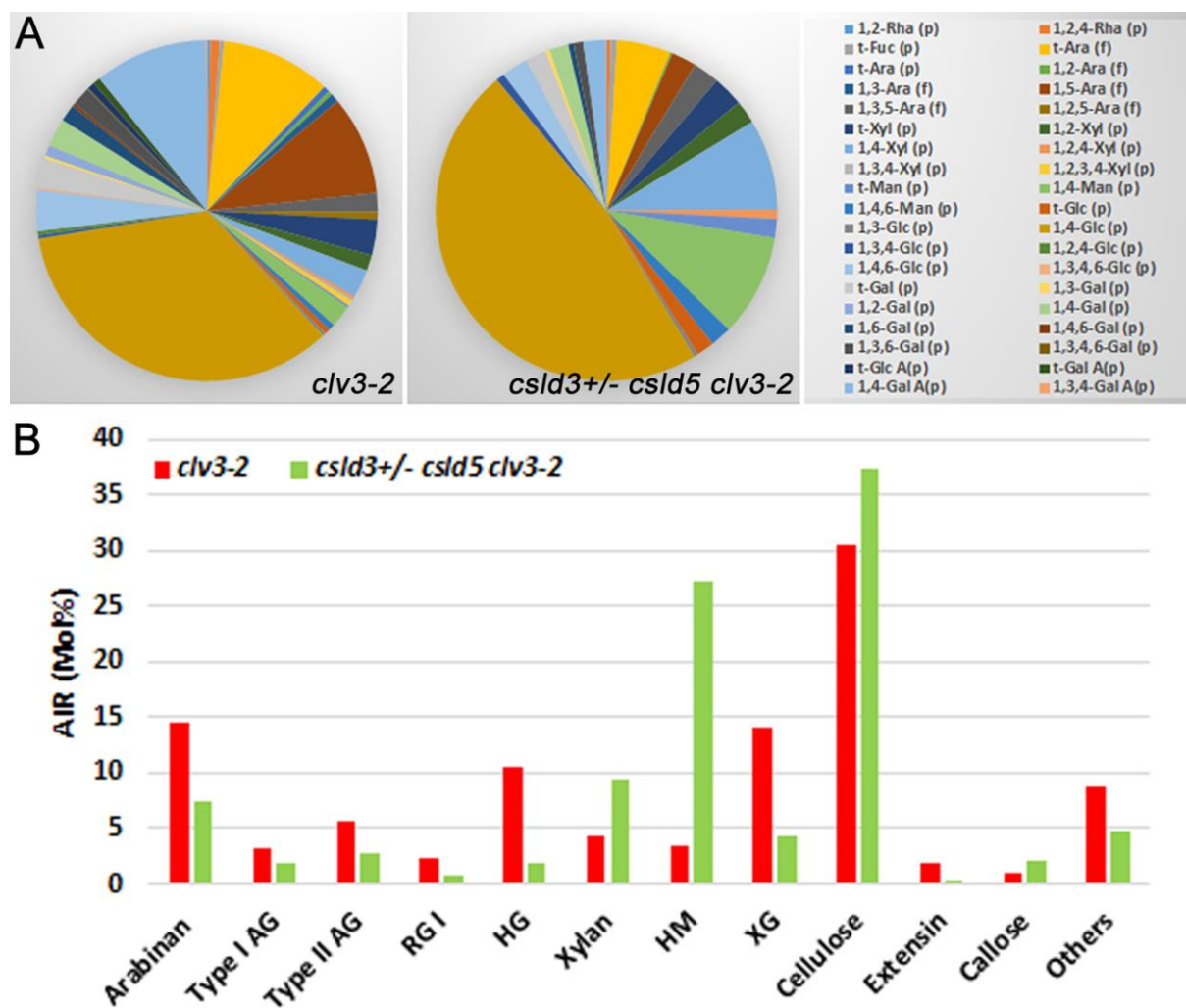
(A) No signals could be detected using sense probes for a selection of GT genes.

(B) The expression patterns of GT genes were not affected by *clv3* mutation. Representative genes of each expression type as shown in Figure 3 were selected to compare their expression in wild-type (Col-0) and *clv3-9* (Col-0 background). All panels are at the same scale, scale bar = 50  $\mu$ m.



**Figure S4. Expression patterns of the transcriptional or translational fusions of GTs in shoot apex. Related to Figure 3.**  
 (A, D) *In situ* hybridization shows the expression of GTs in SAM and flower primordia.  
 (B-C, E-F) Visualization of GT gene expression patterns via translational fusion (B, C) or transcriptional fusion (E, F) in transgenic plants. The vascular initials in (B) are marked with dissolved circle line. Scale bars = 50  $\mu$ m.





**Figure S5. Comparison of polysaccharide composition between *clv3* and *csld clv3* SAMs. Related to Figure 4.**

(A) Chart showing relative quantities of individual linkages in each type of wall. Note that *csld3 csld5 clv3-2* did not give rise to an inflorescence SAM. Instead plants heterozygous for *CSLD3* (*CSLD3/csld3 csld5 clv3-2*) yielded SAMs from growth-retarded plants that were harvested for linkage analysis.

(B) Bar chart showing calculated polysaccharide content in each wall type.

## Supplemental Experimental Procedures

### Calculation of number of wild type meristems for linkage analysis

The upper part of the meristem dome can be assumed to be approximately 15  $\mu\text{m}$  from the top of a spherical cap of radius 50  $\mu\text{m}$ . Assuming the volume of this region, 32000  $\mu\text{m}^3$ , weighs 32 ng, it would require  $1.26 \times 10^5$  dissected SAMs to generate the 4 mg fresh weight to generate approximately 1 mg of cell wall preparation (alcohol insoluble residue; AIR) required for reliable polysaccharide linkage analysis.

### Generating sufficient meristematic material

The above figures illustrate that harvesting from carefully dissected WT meristems is not feasible. We made use of the enlarged, stem-cell enriched SAMs of the *clv3* mutant (Figure S1). Columbia genetic background *clv3* mutant apices have very thin, fasciated SAMs lined with numerous small overhanging flowers making dissection difficult and we were unable to harvest enough cell wall material. However, for the Landsberg *erecta* background the *clv3-2* mutant yields greatly enlarged SAMs of minimum 1 mm radius with developing flowers arranged around the periphery (Figure S1). After minimal dissection, a thin slice of apical tissue (SAM sample) is harvested in the absence of young floral tissue. The enlarged *clv3-2* SAM is enriched in central zone (stem cells) and rib meristem tissue [S1]. In our hands, sixty plants yield at least 1 mg of cell walls (AIR). Young *clv3-2* flowers (stage 6-7) were collected independently to represent a developing and rapidly growing tissue sample (floral sample). Samples were then prepared for either polysaccharide linkage analysis (AIR) or transcriptomics.

### Validation of transcriptome data and data clustering based on gene ontology definitions

Graphical representations of examples of mapped reads that peak either in the SAM, flower or pooled sample are given in Figure S2A. To provide validation i.e. to check the RNA samples are of sufficient quality to mine for expressed GTs, known transcripts with expected enrichment in a sample were examined. Transcription factors controlling floral patterning such as *AGAMOUS* [S2] and *APETALA1* [S3] are enriched in the *clv3-2* floral samples whereas *PIF4/SRL2* [S4] and *PUMILIO10* [S5], genes that have been shown to play a role in the SAM, exhibit expected *clv3-2* SAM enrichment. The root-hair specific *EXP7* [S6] is detected in trace amounts only in whole plant tissues and *TED6* [S7], *CESA7* [S8], *COBL4* [S9], *MYB46* [S10], *LAC11* [S11], *SND2* [S12], expressed during xylem development, are also enriched in the whole plant sample (Figure S2A).

### Normalizing read counts



The total number of mappable reads varied slightly among the six samples and details are given as follows:

	<i>clv3-2</i> SAM	<i>clv3-2</i> floral tissue	<i>clv3-2</i> pooled tissue
<b>No. of biological replicates</b>	2	2	2
<b>Total No. of raw reads</b>	28742265/41004746	35522653/33809171	30268068/30110535
<b>Genome coverage</b>	28.76/40.06	37.24/36.20	20.45/20.95
<b>Mean insert sizes (bp)</b>	237.89/240.18	243.14/240.14	234.32/236.40

In order to visualize mapped reads of different libraries side-by-side we normalized the read counts in each sample with the average genome-wide coverage per bp, and visualised using the Integrative Genome Viewer (IGV) [S13].

Only genes with TPM values > 1 were included in the subsequent analyses. Expression estimates of replicate experiments were averaged and the fold change between the tissue-specific samples and the whole plant sample was calculated for every gene. Subsequently, a Z-score for each fold change was generated analogous to Busch et al., 2010 [S14]. For Figure S2C, transcripts that displayed a fold change > 1.5 and a Z-score greater than 2 were considered as increased, and transcripts with a fold change > 1.5 and a Z-score smaller than -2 were considered as decreased.

### Gene Ontology Analysis

Gene ontology (GO) enrichment analysis was performed using BINGO [S15] implemented in Cytoscape 3.0 [S16]. A hypergeometric test was conducted and the Benjamini & Hochberg False Discovery Rate (FDR) was calculated. A list of GO categories was retrieved containing enriched terms with a p value lower than 0.05 after FDR correction.

### Construction of a pairwise correlation coefficient network

All GT transcripts present at levels >10 TPM were used as a query list for the CORNET co-expression tool [S17] using method “Pearson” and the output showing pairwise correlations for values above 0.5 (Figure S2D). A subset of data series, totalling 958 experiments, were used to generate the network as follows: Development, Flower, Leaf, Compendium 1 and Whole plant.

### Two-colour fluorescent *in situ* hybridization

Processed sections were hybridized with a mixture of two gene-specific probes labelled with digoxigenin and fluorescein, respectively. For detection of digoxigenin labelled probe, the sections were incubated with anti-digoxigenin antibody conjugated to horseradish peroxidase (Anti-DIG-POD, Roche). Hybridization signal was detected with Tyramide Signal Amplification (TSA) System (PerkinElmer). After the first TSA reaction, 3% H<sub>2</sub>O<sub>2</sub> (Sigma) was applied to quench peroxidase activity. The sections

were processed with anti-fluorescein antibody binding (Anti-FITC-POD, Roche) and subsequently TSA reaction. Images were taken with a Zeiss LSM700 confocal equipped with a 20x 0.8NA dry objective. For colocalization analysis with *CSLD5*, the Manders' coefficient M1, representing the fraction of the GT signal that overlaps with the *CyclinB1;1* signal, were determined using the JACoP plugin of ImageJ [S18]. To test the degree of localisation with the expressed *GALS2* signal, the Manders coefficient M2 (fraction of cell cycle marker that overlaps with *GALS2*) was used. The lower cut-off for determining values was a pixel intensity of 35.

### **Making DNA constructs for visualisation of *Atlg32930* and *GATL6* expression**

A 3,145 bp region upstream of the start of the *Atlg32930* open reading frame was amplified using primers PGT31a\_f (5'-GCCGCGGCCGCGTTTAAACttcttcgacacaaatcacacagtgtag) that incorporates both NotI-PmeI restriction sites at the 5' end and PGT31a\_r (5'-GCCGCGCGGCCcttttcttaactcttttcgaa) that incorporates an AscI restriction site. After PCR, the promoter fragment was cloned in to a NotI-AscI digested vector containing a polylinker with NotI and AscI restriction sites, followed 3' by a Gateway recombination cassette and then 3' by the OCS terminator from pBJ36 with a PmeI restriction site at the 3' end. A PmeI fragment containing the promoter::Gateway-OCS sequence was transferred to the PmeI-digested binary vector pMoA34 to generate the destination vector pM34\_GT31aGW. The tdTomato gene fused in frame to a nuclear localization sequence N7 and cloned within a Gateway entry vector was used in a recombination reaction with the pM34\_GT31aGW destination vector. The construct was validated by DNA sequencing and transformed into Arabidopsis plants containing an acylated YFP (myr-YFP) that labels the plasma membrane.

For *GFP-GATL6*, a 3 kb fragment upstream of *GATL6* start codon was amplified using primers GATL6\_p\_F (5'CTGCAGTCTCTTCGTCCGCCTTTG3') and GATL6\_p\_R (5'AAGCTTCGAAACCCTAGAAATAAC3'). The *GFP* coding sequence was amplified using primer EGFP\_F (5'AAGCTTATGGTGAGCAAGGGCGAG3') and EGFP\_R (5'ATCGATCTTGTACAGCTCGTCCAT 3'). A 3 kb fragment containing *GATL6* genomic region as well as 1.6 kb 3' UTR was amplified using primers GATL6\_DNA\_F (5'ATCGATATGCTTTGGATAACGAGATT 3') and GATL6\_DNA\_R ('GTCGACGAAGCTCGACCTCTTTCTTC'). The three fragments were digested with *Pst*I, *Hind*III (*GATL6*\_promoter), *Hind*III, *Cla*I (*EGFP*) *Cla*I, *Sal*I (*GATL6*\_DNA) and then ligated in a standard cloning vector. The whole DNA construct, isolated as a *Pst*I/*Sal*I fragment, was ligated into the binary vector pCambia1300, giving rise to the construct *pGATL6::EGFP-GATL6* and transformed into Arabidopsis. After selection and propagation, plants were subject to confocal microscopy.

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